Gastrulation in Birds.

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In the twentieth volume of the 'Journal of Morphology' a paper by Mr. J. T. Patterson appeared during the year 1909 under the title, "Gastrulation in the Pigeon's Egg: A Morphological and Experimental Study," a preliminary notice of which was published in 1907 in the 'Biological Bulletin,' vol. xiii.

In these papers the author gave an entirely novel account of the process of gastrulation in a bird, which account, if free from error, described an interesting, albeit perplexing, phenomenon.

The paper is fully illustrated by photographs and diagrams, and has the appearance of being a careful piece of work, and it has been used by Professor Frank R. Lillie as the basis of his description of the early stages of bird development in his recent book, 'The Development of the Chick.' Professor Lillie writes, on page 52, that he "has had the opportunity of following the work step by step, and is convinced of its accuracy."

The paper describes so unusual a process that, in spite of this testimony, it courts a rather close examination. Moreover, if correct the matter should be relieved of all suspicion, because it would in that case be a highly important contribution to the embryology of birds.

Since it seems to me that the description given by

Patterson is not altogether free from doubt, I venture to offer the following notes by way of criticism, which may possibly, and I hope will be, successfully met. Briefly stated Patterson's account is as follows: He denies that gastrulation in the pigeon's egg occurs by delamination or any process of ingrowth from the germinal wall or other lower layer segments. Gastrulation, according to him, takes place before the egg is laid by a process of involution of the outermost layer of cells of the segmented blastodisc. At the close of segmentation this outermost layer of cells, which forms a continuous membrane, becomes detached from the sub-lying cells and yolk along that part of its margin which is towards the future posterior end, and the detached margin becoming involuted, grows forward as a thin free edge beneath all the loose cells which admittedly exist in the deeper parts of the segmented blastodisc. This free edge joins up in front and at the sides with the wall of yolk that contains nuclei (i.e. the germinal wall), and forms a continuous sheet of cells—the entoderm or hypoblast. As the subgerminal cavity expands it excavates the germinal wall, and a sheet of cells derived from the germinal wall is left above the cavity. To this sheet the "invaginated" entoderm fuses. Thus the gut entoderm is formed by involution and the yolk sac entoderm by excavation. The loose cells lying beneath the outer layer, now to be termed "epiblast," are said to pass into the outer layer, and so also to form part of the epiblast. This involution process is said to be still further complicated by the concrescence of the lip thus formed giving rise to a linear seam—the future primitive streak, which is withdrawn later within the area pellucida by a sweeping round of the germinal wall in a manner reminiscent of Duval's attempt to prove a process of concrescence at a time subsequent to the laying of the egg.

Critical Notes.

It is claimed that this account of the formation of the entoderm or hypoblast by an infolding of the blastoderm

edge is supported by experimental observations, such as marking certain parts of the blastoderm by injury, and following such marks through several hours of incubation.

Although the account given forms a very complete story, which, if the observations are good, does seem to be supported by a good deal of evidence, yet it is very difficult to reconcile it with the process of gastrulation in the other Amniota. In fact Patterson himself hardly mentions the reptiles or mammals, but confines his efforts to an attempt to adapt the bird to Amphibians and fishes, and more especially to the Teleostean fishes. That is to say, he tries to connect birds with a group far removed from them in anatomical features, and ignores the difficulties presented by his theory when compared with the most closely allied forms.

In no other group of vertebrates is the dorsal lip of the blastopore, which is in every other case the most bulky and actively proliferating part of the embryo, known to exist as a thin or f r e e e d g e ! It is extremely difficult to conceive of the mechanism by means of which such an involution could take place.

Again, on pp. 86–87, the author speaks of the whole thin edge as the dorsal lip of the blastopore, and the yolk as the ventral lip. Now this is never the case in any vertebrate, whether we consider the meroblastic eggs of the Elasmobranch or Teleost or the less heavily yolked eggs of the Amphibia. In all cases the ventral lip of the blastopore, if formed, is a thickened curved rim which is formed in conjunction with the inflection of the epiblast. If Patterson is right in calling the inflected edge of the blastoderm the dorsal lip of the blastopore, then the part which he calls the ventral lip of the blastopore is surely the floor of the gut corresponding to the yolk-plug in Rana. The yolk is never the lip of the blastopore.

Stronger evidence is the table given (p. 90) of measurements made upon living blastoderms during the time supposed to be taken for the process of gastrulation. If the edge of the ectoderm is inflected, one might expect to find a

diminution of the length of the blastoderm occurring at that moment. This is said to have been so in two eggs which were kept under observation for $3\frac{1}{2}$ and $3\frac{1}{4}$ hours respectively.

Patterson objects to some experiments made by myself in 1896 ('Proc. Roy. Soc.,' vol. lx, 1896) with a view to testing Duval's theory of concrescence on chicks, thus: (1) They were performed after concrescence had occurred, which is a valid objection if Patterson's contention that all this occurs before laying is correct. (2) The cells may have flowed round the bristle held in place by vitelline membrane and yolk.

This objection, which, of course, is irrelevant in this particular case if the first holds good, must be considered as a general objection to the use of bristles for such purposes. The bristle, it may be said, makes a perfectly unmistakable landmark, which cannot be said of injuries by canterisation.

The objection is one which has naturally occurred to me, but I am convinced that the objection is groundless for the following reasons:

In the numerous experiments made upon chick and frogs' eggs with sable hairs, I have never seen any evidence that cells can flow round the hair.

The results would not be so constant if there were any flowing of cells round the bristle.

If cells could move so easily as to avoid a bristle without making any visible sign of disturbance, they would be affected by the force of gravity and become displaced when eggs are not in their normal position. This is not the case. A fully segmented egg of Rana temporaria may be held down at an angle of 90° to its normal position without affecting the normal relation of its cells to one another. When there has been a very severe drag upon the bristle on account of some excessive stress in an egg, due to some displacement with reference to the vitelline membrane, and an attempt has been made by the cells to flow round the bristle, the effect is obvious, and is seen as a bay or wrinkle which is quite absent from properly performed experiments of the kind, and indicates only an attempt of a soft tissue to swing round the

obstacle, and is not in any way comparable to an actual flowing of a fluid past a fixed and solid object.[1]

If the segmented egg of a frog, or the segmented blastodisc of a bird, were perfect fluids, then the objection would be a fatal one. Or if the segmented egg were like a heap of shot, then also such a mass could flow slowly past a fixed object without producing any visible rippling. But the segmented ovum is not a pile of separated cells. The cells, or many of them, are in continuity by means of viscous cytoplasmic strands, and the whole mass is by no means a perfect fluid.

If a bristle is inserted into the yolk-plug of an egg of Rana temporaria during the crescent stage of the blastopore close up against the advancing dorsal lip of the blastopore, there is no tendency either for the advancing lip to be divided by the bristle, nor for the bristle to be driven through the yolk plug-cells, thus proving the absence of anything approaching a perfect fluidity of either the ectodermal or the endodermal layer of cells.

I may remark here that when one of my experiments suits Patterson's purpose he accepts it! (p. 115). If cells can sweep past to concresce when the bristle is placed to one side, the fact that my bristle, when placed in the area opaca in the posterior margin, did not appear in the embryo, is no proof that the cells that do form the embryo have not come sweeping past the bristle.

Personally I cannot agree with Patterson's view (p. 109) that concrescence and gastrulation are different phases of the same process. Gastrulation is the formation of the gut cavity. If this formation is accompanied by the production of a blastopore (which is by no means always the case, e.g. Hydrozoa, probably all mammals—I would even add all

[1] These remarks refer to Rana temporaria only. There is much variation in the viscosity of amphibian eggs. I have failed with the segmented egg of Triton cristata. The eggs of Bufo are also less suitable than those of R. temporaria owing to greater fluidity. The results obtained from the chick and Rana temporaria I believe to be quite reliable.

Amniota), then that blastopore may close by concrescence, but the two processes are entirely different phenomena.

I have myself tried for years to emphasise this difference ('94, '96, '08, '09), and the difference is recognised by many embryologists such as Hertwig, Hubrecht, Keibel, MacBride, although they do not use the terms which I humbly protest do most correctly express the essence of the process, namely protogenesis and deuterogenesis. The phenomenon of gastrulation or the formation of the primitive gut-cavity or archenteron, whether with or without a blastopore, is protogenetic, and represents a more ancient phase of evolution. The subsequent phenomenon of deuterogenesis is growth in length and is post-gastrula, and in those animals which have a blastopore formed in connection with the first appearance of gut-cavity it involves all the changes by which the blastopore becomes wholly or partially closed, whether by coalescence, convergence, or concrescence, partial or total. It represents a stage in evolution subsequent to that represented by the gastrula stage.

If there is any concrescence it is concerned with deuterogenesis in the vertebrates and not with gastrulation ; but it is extremely doubtful, in spite of Patterson's work, whether there is any such thing as concrescence in the sense which can be interpreted as meaning that the embryo of the vertebrate is formed by the fusion of the lips of an elongated blastopore.

Patterson adheres with patriotic tenacity to the view so commonly held by Americans as to the formation of the vertebrate embryo by concrescence. He writes thus on p. 103: "In other words, in the teleost the entire margin of the blastoderm separates from the periblast, and this entire margin (germ-ring) concresces to form the embryo." He was presumably unaware of Kopsch's work on the eggs of Salmo, 1905, or he could not possibly have written so dogmatically. Kopsch's experiments prove as conclusively (so it seems to me) as anything can be proved that in the trout the main dorsal axis of the embryo is not formed by concrescence.

From these experiments it is perfectly plain that the germ ring representing the lips of a posteriorly placed blastopore provides the material for growth in length thus—the mid-dorsal part for the mid-dorsal region of the embryo, the nerve-cord and notochord, the lateral parts for the sides, the ventral part for the ventral surfaces. I may refer the reader to some remarks on this in my paper on Teleostean development, 'Guy's Hospital Reports,' vol. lxi, 1907.

If, therefore, Patterson's account of the formation of the main axis of the pigeon by concrescence is correct it is interesting and remarkable, but at any rate it is not like the Teleostean.

Again, where in the animals most closely connected with the birds in adult characters, the reptiles and mammals, can we possibly find the slightest hint of any process either of an involution of a free edge or a process of concrescence?

If we turn from such general considerations to his actual experiments we are not convinced by them.

In the first place there is some, but not much, chance of mistake in the orientation. Patterson says that in the pigeon's egg the embryo lies with its longitudinal axis at an angle of 45° with the longitudinal axis of the egg ("chalazal axis") in 90 per cent. of eggs. Presumably he discarded experiments in which on the development of the embryo it was found to deviate from 45°.

Exp. I. (Operation 33¼ hours, examination 37 hours after the estimated time of fertilisation.)

The posterior margin of the blastoderm, at this time a free edge, was injured by cauterisation before it had become involuted, which injury "ought to be carried down beneath the blastoderm during the course of further development, that is, it ought to be found in the entoderm" (p. 88).

The truth of this contention is supposed to be demonstrated by a photograph (fig. 66). There is nothing to indicate which is anterior or posterior end, but I take it that the number "66" is close to where the edge of the blastoderm should be, and that the space under the letters "op" represents the deficiency in the entoderm. We are asked to compare

this with a section of "an uninjured blastoderm at a corresponding stage," and to note that "the entoderm in this region is very thick (see fig. 37). It is clear, therefore, that while such an operation destroys most of the cells that are to give rise to the entoderm, yet the posterior margin is still capable of forming a rounded dorsal lip." I venture to submit that it is perfectly impossible to deduce any such conclusion from the figures given.

Fig. 66 represents a magnification of 125 diameters, and the point of injury is about $3\frac{1}{2}$ in. from the dorsal lip. Fig. 37 is magnified 245 times, but as the whole section measures less than 5 in. it cannot contain the required spot. There is, however, another figure of the same section, fig. 35, which is magnified 107 times. If we examine the region $2\frac{1}{2}$ in. or even 2 in. to the left of the edge of the blastoderm, we fail to see any greater accumulation of entoderm cells in the uninjured than in the injured one.

Possibly I may have made a mistake in my interpretation of his fig. 66, and the number "66" is at the anterior end and not the posterior end as I assumed. In that case I am at a loss to find either the cells which have been injured or the deficiency in the entoderm referred to. If the latter is indicated by the clearer spot near a letter "z" (of the figure above) then the corresponding spot in fig. 35 or 37 is just as devoid of entoderm as in 66. Or if, as he seems to suggest, we are to contrast fig. 67 with a part still further to the left in fig. 35, I fail to see much difference in the condition of the "entoderm." On this latter assumption, the spot labelled "op" is presumably the "break" in the vitelline membrane made by the operating needle, from which the free edge has curled away forwards. Since there is not a trace of vitelline membrane shown, the photograph fails to strengthen the argument in the text.

I think it must be admitted that the author has not been successful here in his attempt at demonstration.

Exp. II. (Operation $35\frac{3}{4}$ hours after fertilisation. Subsequent incubation 49 hours.)

The injury was again on the edge, but now the edge is a lip. In these he finds an injury in entoderm only, therefore he says there is still an inrolling of entoderm.

In specimens "slightly older" such experiments show injuries in ectoderm and mesoderm but not in the entoderm, "showing that the involution has ceased."

The whole series of experiments recorded under the heading "Experiment II" seems to me to be questionable in the extreme. Anyone reading the first two paragraphs of that section with a critical mind must perceive how fragile is the evidence upon which such far-reaching results are based. He writes (p. 93), in describing the subsequent effect of an injury made to the edge of the lip in the middle dorsal line, "There is no evidence of an injury either in the ectoderm or mesoderm, and hence we must conclude that the affected cells have been brought to their present position (in the entoderm) by an inrolling under the posterior margin. Although this operation has been repeated several times with the above results, yet the position of the injury in the entoderm may vary in an anterior posterior direction; but this variation is easily accounted for by the fact that one can tell in the living egg only approximately the extent to which invagination has progressed."

Thus we see the results obtained are variable; and he goes on to say that "if an injury be made in the same manner as above on slightly older blastoderms, the affected region is not found in the entoderm, but in the ectoderm and mesoderm, showing that the involution has ceased" (p. 93).

There is little here in the nature of exact or accurate experiment. There are no times[1] or measurements given, but instead of these he bases results upon operations performed on "slightly older" blastoderms than those the stage of development of which "one can tell only approximately."

There is also the difficulty presented by this hypothesis of formation of a blastopore lip before the laying of the egg,

[1] Some times are given in the explanation of the plates.

that there would then be a very striking difference compared with other vertebrates. In all other vertebrates the blastopore lip is the growing point for growth in length, and growth in length begins at once, therefore showing itself clearly in the origin of denterogenetic (peristomial) mesoderm from the angle of the lip laterally, notochord dorsally, and deuterogenetic epiblast superficially. This condition is well known not to occur until some hours later—in the chick about the twelfth to fifteenth hour of incubation. So we should have to account for a very remarkable disappearance and reappearance of this proliferating centre.

Again, Patterson in his second series of experiments says that an injury made during the involution process is found in the entoderm, posterior to the position of the nineteeth pair of mesoblastic somites. We are faced with the following dilemma. We can hardly have a proliferating blastopore lip formed as in other vertebrates so long as the outer layer is turning in to form entoderm. Therefore this proliferating lip must come into being after the cessation of that process. Any injuries made to the involuting membrane must surely occur in front of, or beyond, all the tissues, mesoderm included, which are produced by the proliferating lip when it comes into being.

But fig. 50 shows such an alleged injury far posterior to the nineteenth pair of somites. The injury ought to be in front of all the primitive streak mesoblast, whereas, according to the figure, there are many somites of mesoblast in front of the injury.

Another argument which is difficult to follow is the suggestion on p. 99 that certain "cavities in the dorsal lip" are the homologues of Kupffer's vesicle. It is surely well enough established that whatever the physiological meaning may be of Kupffer's vesicle in Teleostean development, it is, as a cavity, part of the gut-cavity. According to Patterson the archenteron is the cavity roofed in by the inturning edge of the blastoderm. Yet here he says that these vacuoles above this roof are homologous to the Kupffer vesicles, which are

well known to be below this roof, i. e. they are part of the archenteron.

Exp. III. (Operation 34⅓ hours after fertilisation. Subsequent incubation 34 hours.)

From the plan of his text-fig. 16 one is bound to conclude that the stage does not materially differ from the stage of the preceding experiment, his text-fig. 10. Each shows a similar diameter, a similar forward extension of the endoderm, a similar width of what he regards as blastopore opening. The only difference is that in text-fig. 10 the dorsal lip of the "blastopore" is slightly convex, in text-fig. 16 slightly concave in surface view.

In Exp. II an injury was made on the edge, and the result was a defect in the endoderm at a spot posterior to the nineteenth somite.

In Exp. III an injury was made on the surface just within the margin. The difference in position of the injury would appear to be not more than the diameter of the needle used. Result, a defect in the region of the head-fold. Therefore the difference in position of less than a needle's diameter in the marking of a blastoderm corresponds with a difference in the embryo which includes the greater part of the body. If this is so we must despair of getting anything approaching accurate results by such methods.

Patterson likewise thinks it unlikely that this small area should give rise to so much embryo directly, and assumes, as we have seen, that there is a concrescence.

Exp. IV. (Operation 34¾ hours after fertilisation. Subsequent incubation for 36¾ hours.)

On a blastoderm similar to Exp. III a spot was marked on the margin 10° to the right of the middle line so close to the margin that the outer surface of the needle was level with it. Result, a defect "on the right neural fold in the mid-brain region."

If the main axis of the embryo is formed by coalescence of the two germ-rings, surely, then, it is in the median plane that the injury should be found, i. e. the ventral wall of the neural

tube and notochord and, perhaps, gut, yet the defect is shown on the upper part of the neural tube only.

Exp. V. (Operation 33⅓ hours after fertilisation, subsequent incubation for 36¾ hours.)

A similar blastoderm was injured at the edge of the horn of the junction zone 45° to the right of the middle line, with the result that a defect is said to have occurred in the primitive streak, though one cannot see much of it in his fig. 71.

In none of the cases so far considered do the figures convince one that the spots called defects are really such, or have any constant relation to the spots injured.

For instance, in the last case it is quite impossible to satisfy oneself that there is any injury at all from fig. 71, and text-fig. 17, which is a transverse section through the alleged injury, shows a perfectly normal primitive streak section with a mass of cells or yolk, or both, lying on the top in no way connected with it. This, in fact, is an "extra-ovate" in Roux's sense that may have travelled from anywhere. My own experiences with such experiments have taught me how deceptive an extra-ovate may be.

The results are very different to the defects figured by Kopsch in his Salmo embryo experiments.

Exp. VI. At a rather later stage—"late gastrular stage"—in which the entoderm had advanced a little further an injury was made at the posterior margin in the median line.

The result was a defect in the middle line at the level of the tenth pair of somites affecting ectoderm only.

Exp. II was supposed to demonstrate the involution of the edge of the blastodisc to form the entoderm, because an injury to the edge made at 35¾ hours appeared only in the entoderm somewhat posterior to the nineteenth pair of somites. In Exp. VI an injury also touching the edge although made three quarters of an hour earlier appeared in the ectoderm only. How can this discrepancy be explained away on Patterson's hypothesis?

Now this one seems open to another explanation. There is clearly an extra-ovate consisting of "a mass of dead cells"

lying between the separated halves of the neural tube. The notochord is perfect and to one side, the entoderm is uninjured. May not the defect in the neural tube be simply mechanical, due to the pressure of an extra-ovate which became separated off from the edge of the blastoderm as a result of the cauterising, and which, passing into the area pellucida between the blastoderm and vitelline membrane, caused the injury seen? Text-fig. 19 strongly suggests this solution.

Exp. VII and VIII. The figures do not enable one to appreciate the character of the defects. Sections are not given.

The remaining experiments, IX–XIII, were made upon the blastoderm after the eggs were laid, and therefore after Patterson's supposed concrescence of the lip must have been completed, because by now, according to him, the main axial line of the embryo produced by this concrescence is entirely enclosed within the blastoderm margin, and there are no longer any free blastoporic lips that could come together.

Although Patterson still speaks of concrescence, Exp. XI, p. 115, it clearly cannot be a phenomenon similar to that which, as he alleges, occurs during gastrulation. One is inclined in this particular connection to say with Professor MacBride (re "Amphioxus," 'Quart. Journ. Micr. Sci.,' vol. liv, p. 302): "Of course in every structure there is an imaginary middle line, and if anyone chooses to say that this band of dividing cells consists of right and left halves which unite together as quickly as they grow, I shall not waste time in arguing against such a metaphysical conception, which is capable neither of proof nor disproof."

From Patterson's final discussion it is clear that he quite fails to appreciate the distinction between gastrulation and subsequent growth in length. It is not really true that "all of the chorda and mesoderm are derived from the primary invaginated layer" in Amphioxus. The anterior part is so derived, but the posterior part is derived from the proliferating lips of the blastopore, which can be described neither as ectoderm nor endoderm.

To me it seems that there is a very great difference between gastral and peristomal mesoblast: the one is protogenetic, the other deuterogenetic.

Nor, again, is it true that in the case of birds the whole mesoblast is formed from the primitive streak. It is altogether difficult to understand why if the primitive streak is formed by the fusion of thickened rims, that thickening should disappear, only to reappear a little later as primitive streak. It is very remarkable and significant of the narrowness of this work that in dealing with avine early stages as compared with other vertebrates the word "reptile" should occur only twice and the "mammal" is mentioned but a single time!

It is pretty evident that the author is utterly unable to reconcile his description (which is an attempt to fit the birds on to fishes) with the facts of reptilian or mammalian embryology, the two groups of animals most nearly connected with the birds.